

Article

# Integrated Management of the Cotton Charcoal Rot Disease Using Biological Agents and Chemical Pesticides

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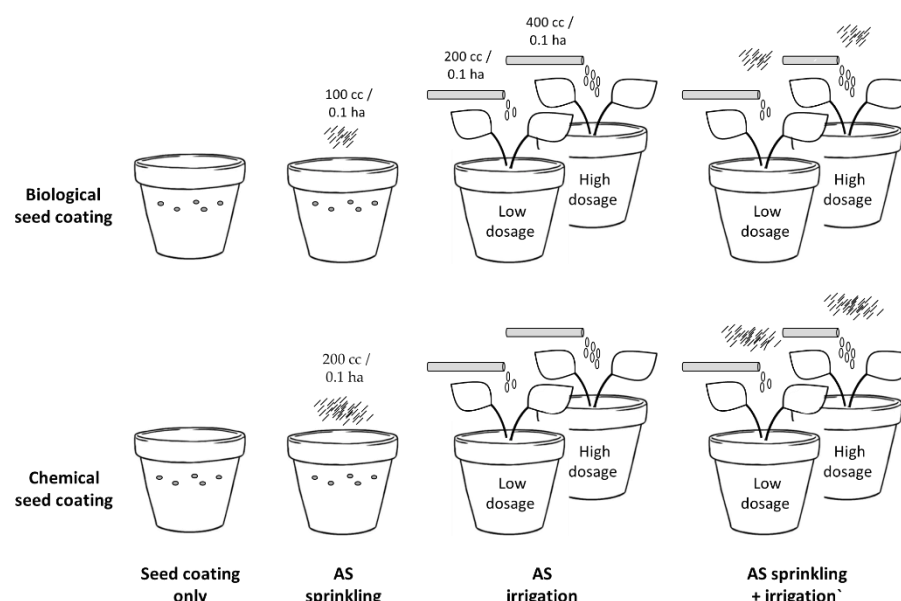
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## Supplementary Materials



**Figure S1.** Experimental design of the pot assay showing the different treatments applied after sowing in substrate infected with *Macrophomina phaseolina*. Two types of seed coating were compared, biological (*Trichoderma* mix) or chemical (a mixture of thiram, captan, carboxin, and metalaxyl-M), solely or combined with the following five different applications of the fungicide Azoxystrobin (AS): by sprinkling at sowing, by irrigation (two dosages) on 10- and 21-day post sowing, or by sprinkling + irrigation. Healthy, non-infected control groups (which also had biological or chemical seed coating) were included. Every experimental group included seven biological replications, for a total of 98 pots.

**Table S1.** Meteorological data for the commercial field experiments <sup>1</sup>.

Parameters	Value
Dates	09/04/2023–07/09/2023
Temperature (2 m above soil, °C)	24.2 ± 6.3
Humidity (%)	67.0 ± 30.8
Radiation (W/m <sup>2</sup> )	294.4 ± 50.9
Precipitation (mm)	81.8

<sup>1</sup> Average data (± standard deviation) according to meteorological station Hulda 24 of The Land Conservation Division, Israel Ministry of Agriculture.

**Table S2.** The plants' growth parameters and survival rate after the different treatments in the pot assay on days 29 and 52 post-sowing, expressed as a percentage of healthy, non-infected controls. <sup>a</sup>

		Day 29 (average / pot)						Day 52 (average / plant)				
	Treatment	Survival (%)	Leaves (no.)	Root length (cm)	Shoot height (cm)	Root weight (g)	Shoot weight (g)	Leaves (no.)	Root length (cm)	Shoot height (cm)	Root weight (g)	Shoot weight (g)
Chemical SC <sup>b</sup>	SC only	106%	98%	111%	95%	97%	98%	116%	102%	114%	122%	131%
	Sp	103%	102%	97%	89%	121%	101%	118%	117%	114%	138%	145%
	D200	100%	93%	90%	105%	90%	93%	107%	108%	113%	128%	118%
	D400	103%	83%	113%	91%	90%	78%	101%	79%	122%	96%	103%
	Sp + D200	91%	65%	76%	68%	72%	51%	91%	84%	95%	114%	91%
	Sp + D400	100%	84%	83%	80%	89%	70%	104%	102%	116%	113%	101%
Biological SC	SC only	97%	106%	94%	97%	94%	114%	110%	110%	110%	137%	134%
	Sp	91%	95%	88%	87%	98%	91%	107%	108%	109%	135%	133%
	D200	94%	95%	98%	92%	78%	91%	109%	120%	116%	129%	122%
	D400	94%	84%	85%	94%	73%	78%	103%	104%	118%	109%	102%
	Sp + D200	91%	85%	75%	95%	71%	85%	97%	111%	112%	100%	105%
	Sp + D400	99%	82%	83%	91%	69%	84%	106%	109%	120%	117%	112%

<sup>a</sup> The treatments were performed once with chemical seed coating (Chemical SC) and once with bio-coating (*Trichoderma* species mix, Biological SC). The treatments are seed coating solely (SC only), seed coating with Azoxystrobin sprinkling (Sp) or irrigation at low (D200) or high (D400) dosage, and a combination of the three (Sp + D200 and Sp + D400). The best and the least influential treatments in controlling the CRD fungus, *Macrophomina phaseolina*, are highlighted in green and pink, respectively. <sup>b</sup> SC – seed coating.

**Table S3.** *Macrophomina phaseolina* DNA in the plants' root after the different treatments in the pot assay on days 29 and 52 post-sowing, expressed as a percentage of healthy, non-infected controls. <sup>a</sup>

		Day 29	Day 52
Chemical SC <sup>b</sup>	SC only	100%	100%
	Sp	38%	49%
	D200	39%	23%
	D400	116%	25%
	Sp + D200	144%	127%
	Sp + D400	280%	66%
Biological SC	SC only	81%	55%
	Sp	49%	35%
	D200	42%	22%
	D400	49%	160%
	Sp + D200	107%	111%
	Sp + D400	163%	263%

<sup>a</sup> The treatments were performed once with chemical seed coating (Chemical SC) and once with bio-coating (*Trichoderma* species mix, Biological SC). The treatments are seed coating solely (SC only), seed coating with Azoxystrobin sprinkling (Sp) or irrigation at low (D200) or high (D400) dosage, and a combination of the three (Sp + D200 and Sp + D400). The best and the least influential treatments in controlling the CRD fungus, *M. phaseolina*, are highlighted in green and pink, respectively.

<sup>b</sup> SC – seed coating.

**Table S4.** Assessment of the yield, symptoms, and *Macrophomina phaseolina* DNA in the plants' roots after the different treatments in the field trial, expressed as a percentage of the control – chemically (Captan) coated seeds plants. <sup>a</sup>

		Yield	Symptoms	qPCR	
Treatment		Day 210	Day 151	Day 74	Day 151
Chemical SC <sup>b</sup>	SC only	100%	100%	100%	100%
	Sp	105%	116%	166%	84%
	D200	101%	156%	140%	115%
	D400	113%	115%	142%	63%
	Sp + D200	101%	148%	131%	123%
	Sp + D400	109%	94%	117%	216%
Biological SC	SC only	117%	94%	128%	78%
	Sp	102%	73%	99%	93%
	D200	104%	127%	122%	97%
	D400	116%	94%	207%	201%
	Sp + D200	98%	126%	135%	127%
	Sp + D400	112%	99%	83%	63%

<sup>a</sup> The treatments were performed once with chemical seed coating (Chemical SC) and once with bio-coating (*Trichoderma* species mix, Biological SC). The treatments are seed coating solely (SC only), seed coating with Azoxystrobin sprinkling (Sp) or irrigation at low (D200) or high (D400) dosage, and a combination of the three (Sp + D200 and Sp + D400). The best and the least influential treatments in controlling the CRD fungus, *M. phaseolina*, are highlighted in green and pink, respectively.

<sup>b</sup> SC – seed coating.